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Quantifying Biochemical Activities in Living Cells with ^{13}C dDNP NMR

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During the last decade, the development of nuclear spin polarization enhanced (hyperpolarized) molecular probes has opened up new opportunities for studying the inner workings of living cells. The hyperpolarized probes are produced *ex situ*, introduced into biological systems and detected with high sensitivity and contrast against background signals using high-resolution NMR spectroscopy, Fig.1.A. Experimental investigations of chemical reactions in the cell require quantitative tools for time-resolved *in situ* analyses in physiologically relevant settings. However, dissolution DNP (dDNP)^[1] is not, a priori, a quantitative method when applied in studies of complex molecular systems and incomplete description of the signal evolution has until now limited dDNP to an observing tool.

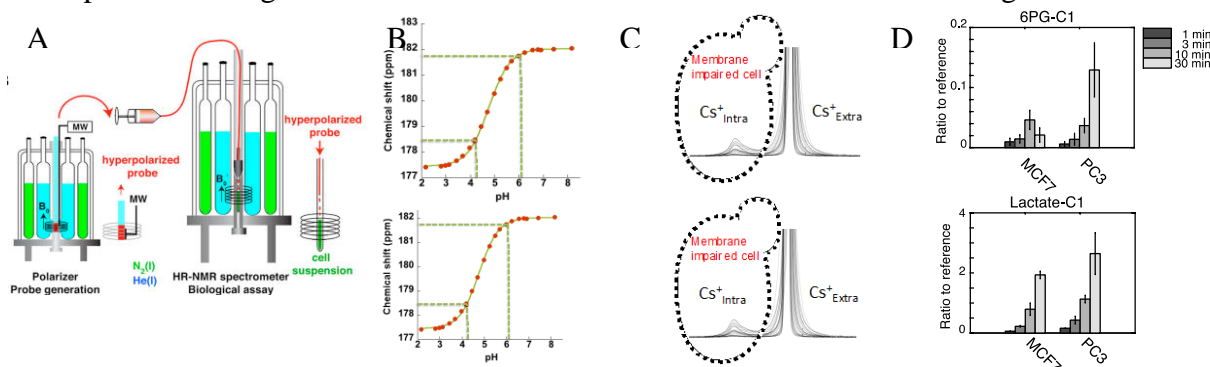


Fig. 1. **A.** Set-up for studying dDNP hyperpolarised bioprobes in living cells. ^[2] **B.** Two compartment system where intra and extra cellular milieu is differentiated by the chemical shift ^[3] **C.** 30% polarisation on ^{13}C s in 5 min allow detection of intra cellular ^{13}C s in membrane impaired cells. **D.** Metabolites (lactate and 6-phospho gluconate, 6-PG) produced over time in breast (MCF7) and prostate (PC3) cancer cells incubated with $^{13}\text{C}_6$ -glucose.

The talk will focus on strategies used for the selection, design and use of hyperpolarized NMR probes in quantitative biological assays. In particular four studies will be discussed:

1) The development of a simple cellular assay from which the intracellular T1 could be measured unambiguously for the carboxylic acid moiety of four compounds. On this basis, a prediction method for the signal evolution in an intracellular environment was developed, Fig.1.B. **2)** Hyperpolarised ^{13}C s NMR has been developed into a sensitive real time biosensor that in seconds provides a background free measure of access to the intracellular environment, Fig.1.C. **3)** Non-persistent photo-induced radicals are shown to efficiently polarise $^{13}\text{C}_6$ -glucose to 50%. This type of radicals can be scavenged through fast thermalisation following DNP^[4] leaving the bioprobe solution free of paramagnetic impurities and consequently with long T1 at appropriate conditions. The storing of frozen hyperpolarised glucose and its continuous thawing for cellular infusion over many minutes is evaluated. **4)** The use of DNP to enhance metabolite extracts benefits from the high sensitivity and resolution provided by hyperpolarized samples in high-field NMR spectrometers, from the possibility to quantify metabolites by comparison with an internal standard, and from the capability to monitor slow metabolic transformations. Biological hypotheses are made using extracts from cancer cells incubated with $^{13}\text{C}_6$ -glucose for many minutes, Fig.1.D.

References

- [1] J. H. Ardenkjær-Larsen et al. *PNAS*, 2003, **100**, 18 [2] M. H. Lerche et al. *Anal. Chem.* 2015, **87** (1), 119
- [3] M. Karlsson et al. *Angew. Chemie*, 2016, **128** (43), 13765 [4] A. Capozzi et al. *Nature Commun.* 2017.